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chemical means to "freeze" the ribosomes engaged in translation at their sites on mRNAs. It should be pointed out, however, that incubation of the cells with cycloheximide for extended periods might lead to an artifactual loading of ribosomes onto mRNAs. Hence cycloheximide should be added to the culture 1–15 min prior to the cell harvest.

f. The gradients should be cold on loading of the sample. They can be prepared at room temperature and then either rapidly cooled by placing them in ice for about 30 min, or kept in the cold room prior to the sample loading. Gradients are known to remain stable overnight.

g. Occasionally, the lysis solution is not clear, due to precipitation of the detergents. It can still be used, but should be mixed well before aliquoting.

h. Heparin is a potent RNase inhibitor, but at high concentrations it might lead to nuclear lysis. To avoid contamination of the cytoplasmic extract the concentration of heparin should not exceed 100 μg/mL prior to the removal of nuclei. Heparin can be substituted, however, by RNA guard (Pharmacia) or RNasin (Promega) at a concentration of 150–300 units mL. These RNase inhibitors require a minimum of 1 mM DTT in the lysis buffer for their activity and should be added directly to the tissues or cells prior to homogenization.

i. SDS is an efficient anti-RNase detergent. However, all steps in which SDS is involved should be carried out at room temperature as this detergent precipitates out of solution at <15°C.

j. Partitioning of the sucrose gradient into two fractions saves time and reagents, particularly if the only relevant parameter is the distribution of an mRNA between polysome and subpolysomes (Fig. 5.1*b*). However, due to the poor resolution of this approach, potentially important information will be lost. Analysis of the distribution of an mRNA along a gradient which is divided into 12 fractions will enable monitoring whether an mRNA is shifted from heavy to light polysomes and allow an estimate of the maximum number of ribosomes loaded on the mRNA (Figs. 5.1*c*, 5.1*d*, 5.2*b*, 5.2*c*, 5.3*b*, and 5.3*c*). The border between the subpolysomal fractions is indicated by the low point in the profile immediately following the polysomal peaks (Figs. 5.1*a*, 5.2*a*, and 5.3*a*).

k. RNA can be isolated by alternative methods as described below.

l. If the polysomes are obtained from a protein-rich source like rat liver, or oocytes and embryos of *Xenopus*, it is often helpful to digest the proteins using the proteinase K/SDS/phenol procedure (Probst et al., 1979). In this case, adjust the method as follows: (1) add 400 μL of PK buffer, instead of the 0.5% SDS buffer; (2) incubate for 30 min at room temperature; and (3) extract the RNA with an equal volume of chloropane as described in steps 30–38, with appropriate volume adjustments.

m. Ideally, RNA will be extracted from all gradient fractions with similar efficiency. This will permit a reliable assessment of the distribution of a specific mRNA among these fractions. However, the large amount of